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STUDIES ON THE REPRODUCIBILITY AND SPECIFICITY OF THE TREPONEMAL IMMOBILIZATION TEST*†

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Since its introduction in 1949, the Treponemal Immobilization Test (TPI) has become accepted as a highly specific test in the treponematoses, although the majority of published reports have dealt with its results in syphilis and in patients suspected of giving non-treponemal reactions with the Standard Tests for Syphilis (STS).

In this paper, the behaviour of the test with non-syphilitic sera is examined to establish the criteria of negativity, its specificity is considered, and the conclusions reached are applied to routine sera.

Technique and Material

The methods used follow those described by Nelson and Mayer (1949) with minor modifications described by Wilkinson (1954). A further modification used at the Royal Free Hospital is the incubation of the tests in a McIntosh and Fildes Jar in an atmosphere of hydrogen 95 per cent. and carbon dioxide 5 per cent. after only one replacement of air with gas mixture.

The sera tested came from the following sources :

(*a*) Antenatal clinics of the Royal Free Hospital.
(*b*) Patients attending the V.D. Department, Royal Free Hospital.
(*c*) Patients attending the Whitechapel Clinic, London Hospital.
(*d*) Patients attending the general departments of the Royal Free Hospital.
(*e*) The second distribution of WHO control serum.

REPRODUCIBILITY

As satisfactory reproducibility is a prime requirement of any laboratory test, this aspect is considered first. Some authors (Olansky, Harris, and Hill, 1953 ; Saurino, 1953) have been dissatisfied with the reproducibility obtained, while Boak, Miller, and Carpenter (1954) achieved good reproducibility by duplicating the test.

The TPI test is liable to technical errors like any other serological test, and therefore should always be repeated on a second specimen of serum before any decision is taken on the results of the test alone.

It has, in addition, certain inherent variables that require comment. First, the antigen is potentially variable in that the treponemal suspension may be partially sensitized with antibody from the rabbit used in its preparation ; secondly, the sensitivity of the test is dependent on the titre of the complement used ; thirdly, a further variation due to sampling error is added during the reading of the test. As there is, therefore, an experimental error, it is important to know how large this error is if the results of the test are to be interpreted correctly.

Results and Discussion

The general behaviour of the test under average conditions is illustrated by Fig. 1, which shows the results in 1,000 consecutive patients in whom a valid result was obtained. In addition, no valid result was obtained with 43 patients due to the specimens being anticomplementary, toxic to treponemes, or infected.

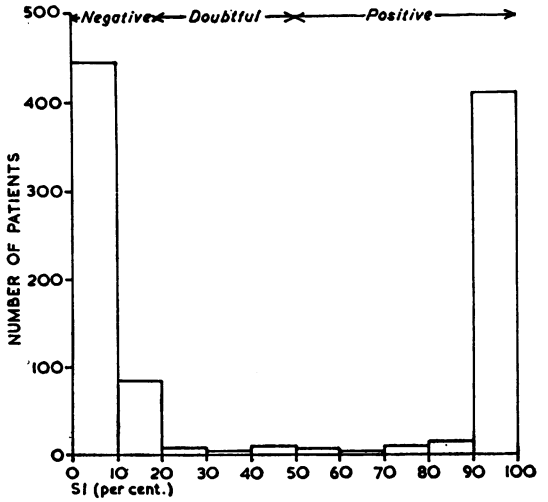

FIG. 1.—Histogram showing results of TPI on 1,000 consecutive patients.

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† Based on a paper read to the M.S.S.V.D. on February 25, 1955.

The value Specific Immobilization (SI) is obtained by substituting the percentage survival in the test and control tubes in the formula given below :

$$\text{Specific Immobilization} = \frac{(C-T)}{C} \times 100 \text{ per cent.},$$

where C = percentage survival in the control tube (no active complement),
and T = percentage survival in the test tube (with active complement).

Inspection of this formula shows that when the test survival is zero (*i.e.*, with a strongly positive serum), the SI will be 100 per cent. irrespective of the control survival, while, in all other cases, the value (C–T) may include the sum of the experimental errors inherent in estimating these two values.

The boundaries between the negative and doubtful, and doubtful and positive zones of 20 per cent. and 50 per cent. SI were laid down by Nelson, Zheutlin, Diesendruck, and Austin (1950) on a basis of experience. Of the 1,000 patients tested, only 22 gave results in the doubtful zone. Of these, three were cases of treated primary syphilis, seven of treated secondary, four of treated latent syphilis, and seven of treated congenital syphilis. The remaining case was a 14-year-old girl whose Kahn test was found to be positive at an antenatal examination. She was treated with penicillin before she was referred to the Royal Free Hospital. Her consort was said to be normal and her STS were negative when examined at the Royal Free Hospital.

Only 33 of the 443 positive patients gave results with an SI between 50 and 90 per cent. ; the majority of the remainder gave an SI of 100 per cent. and were probably of high titre.

Among the negatives, 450 had SIs of 9 per cent. or less and 83 of 10 to 19 per cent. Thus among cases classed as negative, about one in six had an SI of 10 per cent. or more. It is therefore important, from both a theoretical and a practical point of view, to decide if these SI values in the upper part of the negative range are due to minimal amounts of antibody or to experimental error. The sera used in the following study are the 320 that did not give positive results in the series of 323 antenatal patients reported later. The reading technique was modified in this experiment in that the result was recorded after counting 25 treponemes instead of counting further samples if the result appeared to approach the doubtful range. This modification permits a statistical analysis of the working of the test that is not possible in the majority of serological tests (Table I). These figures show the frequencies with which the indicated survivals were observed, *e.g.*, a test survival of 23 treponemes with a control survival of 24 treponemes occurred 25 times, and a control survival of 23 treponemes occurred in all 102 times.

TABLE I

FREQUENCY TABLE SHOWING RELATION OF TEST AND CONTROL SURVIVAL IN 365 TESTS ON 320 NEGATIVE SERA*

No. of Motile Treponemes		Tests											Total
		25	24	23	22	21	20	19	18	17	16	15	
CONTROLS	25	3	10	7	5	4	5	1	3	—	—	—	38
	24	6	19	25	7	6	8	2	3	—	—	—	76
	23	9	17	27	16	12	14	4	1	2	—	—	102
	22	—	3	18	12	12	6	3	3	1	—	—	58
	21	2	3	7	11	7	8	3	2	1	—	1	45
	20	—	—	1	4	6	7	2	2	—	—	—	22
	19	—	1	—	1	3	2	4	1	1	—	—	13
	18	—	—	2	2	—	3	2	—	—	2	—	11
Total		20	53	87	58	50	53	21	15	5	2	1	365

Number	Tests	Controls
Mean Survival	21·8 (87·2 per cent.)	22·54 (90·2 per cent.)
Standard Deviation	1·95	1·78
Sample Variance :		
Sampling Error	2·92	2·22
Variation in Sera or Medium	0·40	0·34
Other Causes	0·48	0·62
Total	3·80	3·18
Standard Error of Variance..	0·28	0·24

* The statistics are given in terms of organisms per sample.

The two causes of variation, which can be calculated from these figures, are that due to sampling error, which depends on the mean survival and the size of the sample examined, and that due to non-specific variations in the medium on different days or in the individual sera resulting in high (or low) survivals occurring together in the same test, which is calculated from the correlation coefficient.* As variance is additive, the variance due to unexplained causes can be found by subtracting the variance of known causes from the observed variance. This remainder will be subject to the errors inherent in the estimations of variance from which it is calculated. The error in estimating the variance of the

* The correlation coefficient, r, is a measure of the relationship between two variables. r may be of any value from $+1$ to -1. When $r = +1$, the variables are directly proportional to each other. When $r = 0$, there is no relationship, and when $r = -1$, the relationship is inverse.
In this example, $r = 0{\cdot}324$.
The variance due to this correlation = $r^2.V$,
where V = the variance.
The variance due to sampling error will equal that of a similar binomial distribution and is given by the following :
Variance of sampling error = $p.q.n$,
where p = proportion motile
q = proportion non-motile
n = number in sample.

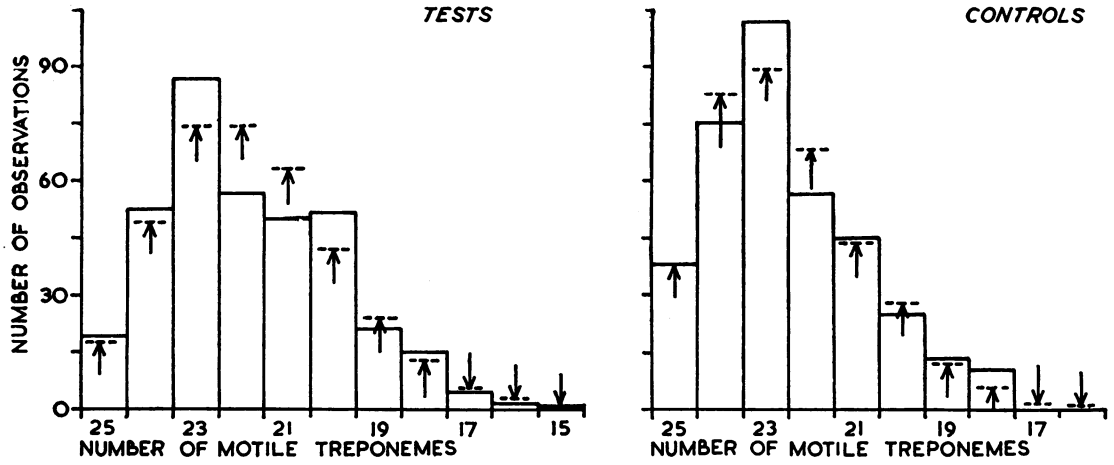

FIG. 2.—Histograms showing survival in tests and controls of 365 TPIs on 320 negative sera. Arrows indicate predicted height of columns.

sampling error is negligible. The standard error of the correlation coefficient is such as to increase the standard error of the variance due to " other causes " from 0·28 (S.E. of variance) to 0·31 in the tests, and from 0·24 to 0·26 in the controls. There is, therefore, a two-thirds chance that the variance from " other causes " lies in the range 0·17–0·79 for the tests, and 0·36–0·88 for the controls (+ or − one S.E.), and a 95 per cent. chance that they do not exceed 1·10 and 1·14 (+ two S.E.) respectively. Thus, in this series, there is no evidence that the tests show a greater unexplained variation than the controls that might be attributed to the presence of an immobilizing antibody in the sera examined.

The lower mean survival in the tests, therefore, may be due to a steady amount of sensitization of the treponemes with antibody from the rabbits used or to a non-specific action of complement.

The hypothesis that the variation observed is due to sampling error superimposed on a much smaller variation in survival in the individual tests or batches can be further examined by comparing the observed distributions with those calculated on the basis of this hypothesis (Fig. 2)*. There is no significant difference between these distributions (tests, p = 0·3 ; controls, p = 0·5).

If this hypothesis is accepted on the agreement between the observed and expected distributions, it would appear that the method of reading the test introduces a greater error than the variation in survival that occurs.

The results of the 365 tests expressed as SIs are shown in Fig. 3 † together with their expected distribution. There is no significant difference between the observed and expected distributions (p = 0·15). Twenty tests have SIs of 20 per cent. or over against 25 predicted.

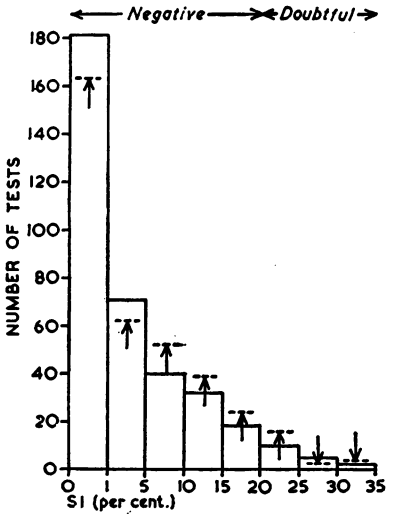

FIG. 3.—Results as specific immobilization of 365 tests on negative sera. Arrows indicate expected height of columns.

When the results of the 45 repeat tests on sera with high values of SI are averaged with the original results, the high values of SI disappear. This and the final results on the 320 sera are shown in Fig. 4 (opposite).

* The expected values shown in Fig. 2 are modified Poisson distributions having means and variances equal to those observed.

† The expected values shown in Fig. 3 are derived from similar distributions but of variance equal to that of the sampling error plus the variance " from other causes ". The expectation of each combination of survivals (*e.g.*, test 92 per cent., control 96 per cent.) was calculated and these were grouped according to SI.

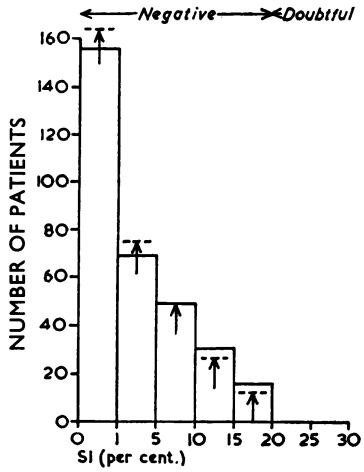

FIG. 4.—Results of 320 negative sera after averaging original and repeat results. Arrows indicate distribution found by Wilkinson (1954) among negative clinic cases (adjusted to same total).

The arrows indicate the distribution of SIs obtained by Wilkinson (1954) on patients attending the Whitechapel Clinic who were presumed to be non-syphilitic. There is no significant difference between the two distributions ($p = 0{\cdot}7$).

These results show that, with negative sera, the reproducibility of the TPI approaches the maximum possible, and that, using similar techniques, the two laboratories obtained almost identical distributions of results using sera from different sources.

Sera giving doubtful results were insufficient for analysis. By examination of the results of repeated quantitative examination of the same positive control serum, the best estimate of the behaviour of the TPI in this zone is obtained. Suitable dilutions of the serum are set up and the SI of each is estimated. From these values, the dilution that would have produced an SI of 50 per cent. is estimated graphically. Fig. 5 shows the results obtained in the two laboratories over a period of about 10 months using the second WHO control serum. A number of invalid estimations are included for comparison, those marked " C " had insufficient residual complement, and those marked " S " were discarded because of sensitization with rabbit antibody. These invalid estimations fell among the lowest (C) and highest (S) titres found. The invalid estimates were not included for calculation of mean titre and standard deviation.

The difference between the mean titres of the two laboratories is less than half a dilution, and the standard deviation in each was between a third and a half dilution. Thus, in quantitative estimations, the majority of results will fall within half a dilution of the mean value and almost all within the generally accepted range of reproducibility of one dilution. The agreement between the two laboratories would also appear to be satisfactory.

The mean of forty estimations of SI of the 1 in 16, 32, and 64 dilutions were 93·7, 54·8, and 20·4 per cent. respectively, indicating that, where partial immobilization occurs, doubling the amount of antibody corresponds to an increase in SI of about 35 per cent.

These results show that while the general degree of reproducibility is satisfactory, the variation that occurs gives results, reported in terms of SI or titres producing SI 50 per cent., a misleading appearance of accuracy unless each test is repeated several times.

The conclusions reached from the laboratory aspect of the test are borne out by the results of repeat tests on the same specimen of serum and on different specimens of serum from the same patient. The results of repeat tests on the same specimen of serum are shown in Table II (overleaf). These repeats were carried out only when the original result appeared to require checking, and they, therefore, represent the worst agreement found among the results of the 1,000 patients shown in Fig. 1. The results are divided into four groups :

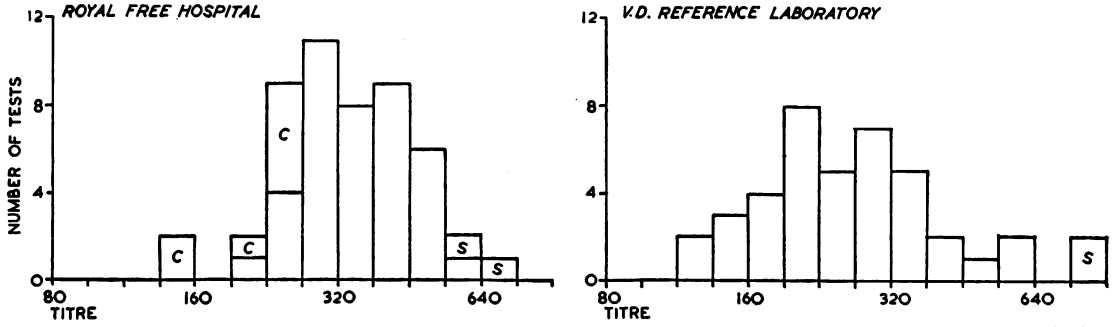

FIG. 5.—Histograms of results of quantitative tests on same serum (invalid estimations : (C) insufficient complement ; (S) sensitization).

TABLE II
RESULTS OF REPEAT TESTS ON 240 SERA SELECTED FROM THE 1,000 SHOWN IN FIG. 1 REQUIRING CONFIRMATION OF RESULT

Second Test		First Test			
		Positive	Weak Positive	Doubtful	Negative
	SI	100–76	75–50	49–20	19–0
Positive ..	100–76	105	3	0	0
Weak Positive	75–50	3	2	3	0
Doubtful	49–20	2	5	8	9
Negative	19–0	0	0	29	71

(*i*) negative (SI, 0–19 per cent.),
(*ii*) doubtful (SI, 20–49 per cent.),
(*iii*) weak positive (SI, 50–75 per cent.),
(*iv*) positive (SI, 76–100 per cent.).

The result of the first test is shown at the top of the Table and the second at the side. Among the 240 pairs of tests shown, complete agreement occurred in 186 pairs (77·5), and minor disagreement (that is, one test negative the other doubtful, one test doubtful the other weak positive, or one test weak positive the other positive) occurred in 52 pairs (21·6 per cent.) of which 38 occurred between doubtful and negative and correspond to the doubtful results shown in Fig. 3. A greater difference was observed in only two sera (strong positive to doubtful), and in both of these the intervening zone was just straddled. These results support the conclusions already reached, and are comparable with the reproducibility obtained with repeat tests on the same specimens of serum with the STS.

The results of tests on more than one serum from the same patient are shown in Table III. Where there were more than two specimens and the results were discrepant, the strongest and weakest results are shown. These, in all cases, were the first and last serum examined.

TABLE III
RESULTS OF REPEAT SERA ON 283 PATIENTS

Last Specimen	First Specimen		
	Positive	Doubtful	Negative
Positive ..	165	2	0
Doubtful ..	4	10	3
Negative ..	Early syphilis treated, 3 Normal infants, 2 Tabes treated, 1 } 6	Early syphilis treated, 4 Latent syphilis treated, 1 I.K., 2 ?Congenital syphilis treated, 1 } 8	85

Among the 283 patients, 260 (92 per cent.) showed complete agreement of results. In five (1·8 per cent.) there was an apparent increase in the strength of the reaction, but no cases changed from negative to positive. In seventeen patients (6 per cent.) there was an apparent decrease in strength, and six of these (2·1 per cent.) changed from positive to negative. These six cases comprised one of treated primary syphilis, two of treated secondary syphilis, and one of treated latent syphilis. In addition, there were two clinically normal infants whose TPI tests were found to be positive after birth, and whose mothers had been treated for latent syphilis during pregnancy. These two cases probably represent a passive transfer of maternal antibody and its gradual disappearance. While immobilizing antibody disappears more slowly than reagin, it does not persist longer than some other antibodies. Serological results of four of these six cases on whom more than two TPI tests were carried out are shown in Figs 6 to 9. The long period between treatment and the change in the TPI test from positive to negative in Fig. 9 suggests that the TPI may show a reduction in titre for a very long period after treatment. In none of the six cases was the change from positive to negative at variance with the clinical findings.

Conclusion

The reproducibility of the TPI, as assessed by the analysis of results obtained with negative sera and

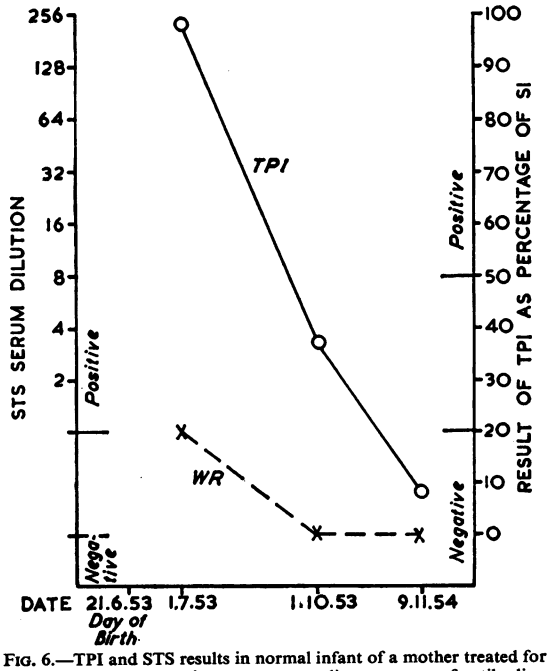

FIG. 6.—TPI and STS results in normal infant of a mother treated for latent syphilis showing spontaneous disappearance of antibodies.

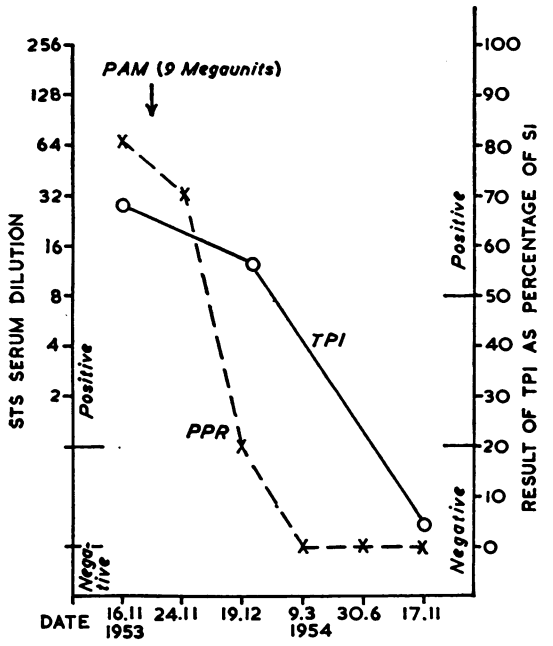

FIG. 7.—TPI and STS results in male, aged 22 yrs, with 2 weeks' history of a genital sore, of treated primary syphilis showing disappearance of antibody following treatment with penicillin.

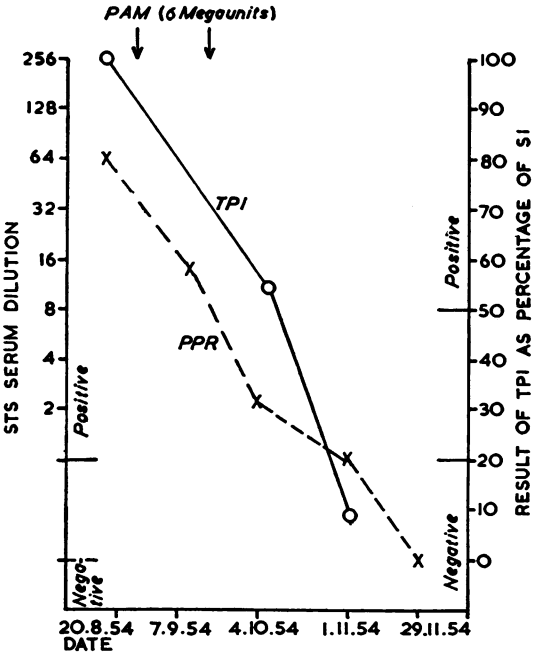

FIG. 8.—TPI and STS results in female, aged 26 yrs, with 2 weeks' history of rash, of secondary syphilis showing disappearance of immobilizing antibody after treatment with penicillin.

positive control serum, is comparable to that of the STS. This conclusion is supported by the results of repeated tests on the same sera and tests on different sera from the same patients.

SPECIFICITY

In the immediate post-war years, the problem of suspected non-treponemal reactions with the STS was acute, especially in the U.S.A. The application of the TPI to this problem, without prolonged assessment of the test, would appear to be justified in that no reported cases with non-treponemal reaction based on confirmed TPI results have developed other evidence of syphilis.

The acceptance of the test as a highly specific one for the treponematoses would, however, appear to be based upon the use of virulent treponemes as the antigen rather than upon experimental evidence : Zellmann (1954) was

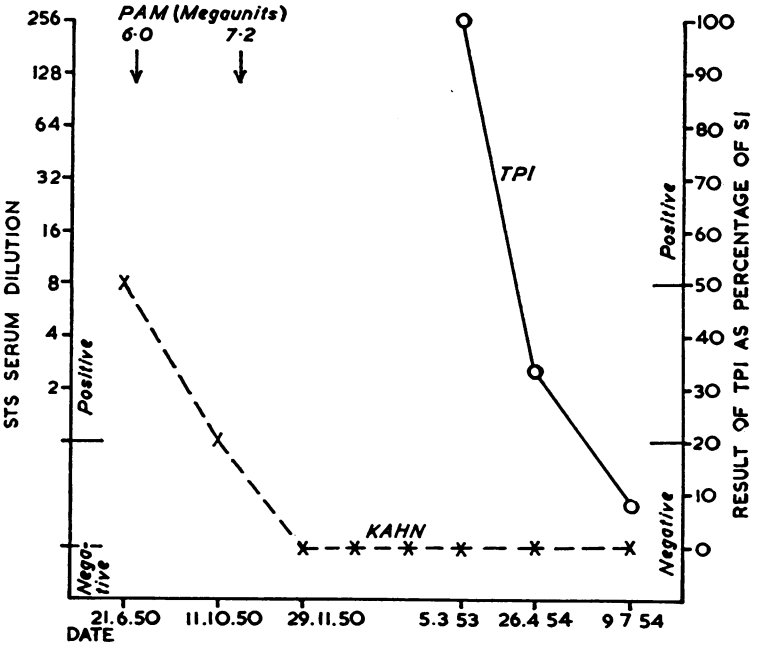

FIG. 9.—TPI and STS results in a case of secondary syphilis showing disappearance of immobilizing antibody 4 yrs after treatment. Female, aged 38 yrs, with history of 3 months' rash and 2 months' hoarseness.

able to collect reports from the literature on only 1,249 non-syphilitic patients to which he added a further 148 cases of his own (total 1,397). Of this total, 289 cases included in the non-syphilitic group would appear to be the same cases as those grouped by Chacko (1953) into 109 normal individuals, 132 cases with diseases other than syphilis, and 48 cases suspected on clinical grounds of giving non-syphilitic reactions with the STS. Excluding these, the total number then reported by Zellmann was 1,108, of which the largest series was 241 patients reported by Chacko.

The TPI would appear to be specific in the immunological sense, that is, based upon a true antigen-antibody reaction, as it consists of an extremely elegant interaction between organism, antibody, and complement. Its specificity in practice will therefore depend on the uniqueness of the antigen and therefore upon the antibody involved. The sharing of antigens by related species is the rule rather than the exception. Well known examples are the treponematoses, the Salmonella, and the viruses of the Lymphogranuloma-Ornithosis group. The sharing of antigens by unrelated species, while not common, is illustrated by the agglutination of strains of *Proteus* by serum from cases of Rickettsial infection (Weil-Felix reaction) and the occurrence of serologically similar capsular antigens in the Type B Friedlander Bacillus and the Type II Pneumococcus. In each of these examples, the shared antigen is a polysaccharide, and, in addition, each organism contains a specific antigen with which no cross-reaction occurs. Hardy and Nell (1955) have shown that the *Treponema pallidum* can be agglutinated by two antibodies, one of which can be absorbed with lipid antigens and is presumably Wassermann reagin, the other being a specific anti-treponemal antibody which may be identical to immobilizing antibody. It seems, therefore, that *Treponema pallidum* contains not only a lipid antigen related to substances occurring widely in nature which forms the basis of the STS, but also one or more specific antigens. The sharing of two dissimilar antigens with unrelated species would appear extremely unlikely.

If the antibody that immobilizes the *Treponema pallidum* in the presence of complement occurs in conditions other than treponemal infections, the antigen that stimulates its production must have a relationship with some factor or disease with which its association might be demonstrated, as has been done with some of the causes of non-treponemal reactions with the STS. The number of non-syphilitic cases so far studied with the TPI is as yet insufficient to demonstrate any such association.

If immobilizing antibody only occurs in treponemal infections, the natural history of these diseases and the extreme persistence of immobilizing antibody make it virtually certain that a proportion of cases with positive TPIs will have no other evidence of treponemal infection. It therefore follows that the absolute estimation of the specificity of the TPI will not be possible for a considerable time.

RESULTS AND DISCUSSION

The sera reported here are divided into three groups :

(*a*) 323 patients attending the antenatal clinics of the Royal Free Hospital. These were selected on the basis of having negative STS.

(*b*) 103 individuals classified as non-syphilitic, comprising 92 patients attending the Royal Free Hospital with diseases other than syphilis, and eleven normal individuals.

(*c*) 345 patients attending the Whitechapel Clinic, London Hospital, who were presumed to be non-syphilitic. (These cases have already been reported by Wilkinson, 1954.)

The results of the TPI in these cases are shown in Table IV, together with the totals collected by Zellmann (1954).

TABLE IV
TOTALS OF PRESUMED NON-SYPHILITIC CASES

Reference and Category	Totals	Result of TPI		
		Positive	Doubtful	Negative
After Zellman (1954)				
Normal	389	0	0	389
Diseases other than syphilis	615	1	1	613
Non-syphilitic	393	2	0	391
Total	1,397	3	1	1,393
Less non-syphilitics of Chacko (1953)	289	2	0	287
CORRECTED TOTALS ..	1,108	1	1	1,106
Wilkinson (1954)				
Clinic cases presumed non-syphilitic	345	10	2	333
Sequeira				
Unselected antenatal patients with negative STS	323	3	0	320
Non-syphilitic	103	0	0	103
Totals	771	13	2	756
Less possible syphilis and defaulters	11	10	1	0
CORRECTED TOTALS ..	760	3	1	756
GRAND TOTALS ..	1,868	4	2	1,862

Brief clinical details of all positive and doubtful cases are shown in Table V. Among 1,879 patients initially presumed to be non-syphilitic, seventeen gave positive or doubtful TPI results. Of these

TABLE V

CASES INITIALLY DIAGNOSED AS NON-SYPHILITIC IN WHOM THE TPI WAS FOUND TO BE POSITIVE OR DOUBTFUL

Reference and Category	TPI Result	(% SI)	Notes
Zellman (1954) 103 cases with diseases other than syphilis	Positive	—	Paroxysmal auricular fibrillation ; no history or signs of syphilis.
	Doubtful	—	Disseminated sclerosis ; no signs or history of syphilis.
Wilkinson (1954) 347 V.D. clinic cases classed as non-syphilitic	Positive	(94)	British West Indian. Emigrated, not available for investigation.
	Positive	(64)	British West Indian. Vague history of yaws in childhood.
	Positive	(60, 60)*	Pakistani. Treated for penile sore with injections at sea in 1954.
	Positive	(100)	British West Indian. "Cut on penis" in 1940. Treated with tablets and injection in Jamaica.
	Doubtful	(45)	British West Indian. Attended with gonorrhoea. Defaulted.
	Positive	(100)	British Guianian. Cardiolipin W.R. positive on same specimen of serum.
	Positive	(64)	History of I.V. injections in V.D. clinic 20 yrs ago.
	Positive	(100)	Cardiolipin W.R. positive on same specimen of serum.
	Positive	(91)	Husband with syphilitic glossitis. Referred as contact of late syphilis.
	Positive	(100)	Prostitute. Defaulted.
	Doubtful	(28)	Venereophobia. Single specimen gave SIs of 48, 19, 33, 12 per cent.
	Positive	(78, 76)*	Venereophobia. Vague history of injections for boils some years previously
Present Series: 323 antenatal cases with negative STS	Positive	(100, 96)*	History of twins dying soon after birth. One child (hydrocephalic) TPI and STS negative.
	Positive	(100)	Austrian. History of I.V. injections in prison camp during war.
	Positive	(100)	Parents and sibling said to be normal. Central incisors missing, "were bad and crooked".

* Two specimens examined.

three were not available for further investigation, while in eight evidence was later found suggesting the possibility of previous treponemal infection. Therefore, among the 1,868 patients finally presumed to be non-syphilitic, four (0·21 per cent.) gave positive and two (0·11 per cent.) gave doubtful reactions. In none of these cases can syphilis be absolutely excluded as in no case was a full epidemiological study carried out. It therefore appears that the occurrence of non-specific reactions with the TPI is not yet conclusively proved.

To assess the possible significance of these TPI positive–STS negative results, it is necessary to consider the behaviour of the TPI in known cases of syphilis, and to examine the incidence of this pattern of results. In early untreated syphilis, the STS become positive before the TPI, practically all cases of untreated secondary syphilis having a positive TPI (Magnuson and Thompson, 1949). In treated early syphilis, the TPI remains positive longer than the STS. The behaviour of the TPI in latent syphilis is discussed elsewhere (Wilkinson and Sequeira, 1955). In late syphilis, the great majority of patients have a positive TPI which appears unaffected by treatment. The few cases with a negative TPI are examples of very long standing congenital or acquired infection.

There are, in addition, certain other sequels that are theoretically possible after infection with the *Treponema pallidum*. The organism might be eliminated, either spontaneously or following the administration of antibiotics for some other condition, without any sign or symptom of syphilis being produced, or the normal manifestations of the disease might be suppressed without the eradication of the infection. In either of these eventualities, production of immobilizing antibody might occur without the production of reagin. That the latter phenomenon can occur in experimental syphilis has been demonstrated by Nelson (1952). In this experiment, he under-treated a group of syphilitic rabbits with penicillin, some of which reacted in the same way as the fully-treated controls. In two, however, the reagin titre fell to negative, while the TPI titre fell and then rose. At the end of the experiment, virulent *Treponemata pallida* were recovered from the lymph glands of these rabbits in spite of the negative STS. The possibility of such cases occurring in man was examined as follows. The TPI was carried out on the sera of patients with gonorrhoea at periods varying from 6 months to a year after treatment with penicillin. Among sixteen such patients, in whom there was no history or clinical evidence of syphilis, one had positive STS and TPI, and one had negative STS and positive TPI. This latter patient was an Englishwoman of 28 years whose gonorrhoea was treated with penicillin and who returned 2 months later with a gonorrhoea re-infection which was again treated with penicillin. Her husband had neither signs nor evidence of syphilis or gonorrhoea. Her TPI was found to be positive 10 months after her first attack of gonorrhoea, and was confirmed with a second specimen. Her STS were negative throughout. These results are inconclusive in that a TPI was not carried out on her first attendance.

That negative STS and positive TPI cases analogous to untreated latent syphilis can occur, is suggested by the finding of five patients with this

pattern of results among fourteen mothers of congenitally syphilitic children (Wilkinson and Sequeira, 1955).

Table VI shows the incidence of positive TPIs associated with negative STS in patients attending the Royal Free Hospital. The totals indicate the total number of sera received by stage of syphilis. In early and latent syphilis, treatment results in a considerable proportion of cases with negative STS and positive TPI. Among patients attending hospital with late symptomatic syphilis, and representing in general a later age group, a proportion have negative STS and positive TPI. Of fifteen untreated patients with cardiovascular or neurosyphilis, three had this pattern of results, one of these having aortic incompetence with radiological appearances supporting a diagnosis of syphilitic aortitis. A later specimen of serum from this patient had a positive Wassermann reaction and a negative Kahn test and Price's precipitation reaction. The other two cases had tabes dorsalis : in one, the condition was of long standing and may no longer have been active, and in the other the cerebrospinal fluid changes indicated an active infection.

TABLE VI
CASES OF SYPHILIS FOUND TO HAVE NEGATIVE STS AND POSITIVE TPI

Diagnosis Syphilis	No. Untreated		No. Treated	
	Total	STS− TPI+	Total	STS− TPI+
Early	13	0	81	16
Latent	—	?	121	28
Of cardiovascular system ..	6	1	6	1
Of central nervous system ..	9	2	22	10
Congenital	0	0	34	3

From these results it appears that the TPI may be positive in association with negative STS in active syphilis. The TPI in patients with no signs or history of syphilis reveals a proportion of positive results. Those with positive STS are treated as latent syphilis. The exact status of those with negative STS is still in doubt, but it would appear probable that a proportion represent active infections.

SUMMARY

The reproducibility of the Treponemal Immobilization Test from a laboratory aspect and in routine use is comparable to that of the STS.

While there are as yet insufficient reports of results on non-syphilitic sera for an absolute assessment of specificity, the results so far available indicate that the TPI is highly specific for the treponematoses. It appears likely that a proportion of patients with negative STS and positive TPI may represent a form of latent syphilis.

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